REMARKS

Applicants respectfully requests entry of the amendments and remarks submitted herein. Claims 1, 11-14, and 18-19 are amended, and claim 7 is canceled. Therefore, claims 1-6, 8-19 are currently pending.

Applicant respectfully submits that the amendments to the claims are supported by the specification as filed and that no new matter has been added. Claim 1 has been amended to recite the features of original claim 7. Claim 11 has been re-written into independent form, reciting the features of original claims 1 and 10. Claims 13, 14, and 18 have been re-written into independent form reciting the features of original claim 1. Claim 19 has been amended to correct a typographical error.

Claim Rejections under 35 U.S.C. §102(b)

The examiner indicated that claims 1-10 are rejected under 35 U.S.C. 102(b) as being anticipated by Viriyakosol et al. (J. Biol. Chem. 2001 276:38044-51).

In order for a cited reference to anticipate a claimed invention under §102, a single reference must disclose each and every limitation of the claimed invention. *Moba, B.V. v. Diamond Automation, Inc.*, 325 F.3d 1306, 66 USPQ2d 1429 (Fed. Cir. 2003).

Claim 1 recites a purified complex comprising endotoxin bound to MD-2, wherein the complex consists essentially of one molecule of endotoxin bound to one molecule of MD-2. Pending claims 2-6 and 8-9 depend directly or indirectly from claim 1.

Viriyakosol et al. discuss the binding of MD-2 to purified rough LPS (*i.e.*, endotoxin) in ELISA assays, fluorescence assays, photoaffinity labeling assays, sucrose density gradient assays and native PAGE assays (Viriyakosol et al. at page 38045). In the ELISA assays, Viriyakosol et al. disclose either binding MD-2 to a 96-well plate and contacting it with biotin-LPS, or binding LPS complexed to BSA to a 96-well plate and contacting it with MD-2. In the fluorescence assays, they mixed FITC-LPS with MD-2. In the photoaffinity labeling assays, ^{125}I-ASD-LPS was incubated in the presence of various concentration so proteins, and analyzed on an SDS-polyacrylamide gel. In the sucrose density gradient assays, [^{3}H]LPS was mixed with MD-2. In the native PAGE assays MD-2 was incubated with Re595 LPS and electrophoresed in a nondenaturing PAGE gel.

In the various Viriyakosol et al. experiments, the LPS was present in the form of an aggregate, and not in the monomeric form, as recited in the pending claims. This is an important difference. Viriyakosol et al. does not specifically state whether the LPS was in an aggregate form. Neither the composition nor the functional activity of the LPS bound to MD-2 reported by Viriyakosol et al. was examined, hence not defined. The conditions used by Viriyakosol et al., however, were similar to those used by Iovine et al. (J. Biol. Chem., 277:7970-7978 (2002) (submitted with IDS on February 14, 2005); and Giardina et al. (J. Biol. Chem., 276:5883-5891 (2001) (submitted with IDS on February 14, 2005)), wherein the endotoxin was shown to be in aggregate form in similar experimental conditions. These papers demonstrate that state of the endotoxin used in Viriyakosol et al. was as aggregates.

It should be further noted that Viriyakosol et al. merely showed that MD-2 interacted with LPS, and not that MD-2:LPS formed a monomeric complex (i.e., one molecule of endotoxin bound to one molecule of MD-2) as recited by the claims. As shown by the present application, the conditions for generation of the monomeric complex are substantially different from the conditions used by Viriyakosol et al., and thus yielded different products. Figure 1 of the present specification demonstrates that single as opposed to aggregate presentation of the endotoxin produces dramatically different results. Please note that the combination of LOSagg with culture medium containing active MD-2 resulted in no cell activation. In contrast, when the inventors incubated LOS:sCD14 with the same culture medium containing sMD-2, the cells were activated. In fact, efficient generation of the monomeric endotoxin:MD-2 complex recited by the claims requires incubation of monomeric endotoxin:CD14 complex with MD-2, and is not achieved by incubation of endotoxin aggregates with MD-2. This shows definitively that interaction that was studied in Viriyakosol et al. does NOT yield bioactive E:MD-2 complex, and thus could not be monomeric MD-2:E complex.

Further, it should be noted that Viriyakosol et al. needed orders of magnitude higher concentration of reactants in order to obtain binding of the aggregated endotoxin to MD-2, as compared to the inventors when generating the claimed monomeric MD-2:endotoxin complex. Scientifically, the reason for this is because of the amphipathic properties of endotoxin. Endotoxin in physiologic buffered salts solutions, even at ng/ml concentrations, exists entirely in aggregate form (as a means to bury the hydrophobic lipid A region from water; much as it does

in the bacterial outer membrane where lipid A is buried within the outer leaflet of the outer membrane).

Moreover, it should be noted that it was surprising and unexpected to the inventors when they discovered that the endotoxin was bound to the MD-2 in a monomeric form. As indicated on page 3, lines 9-11 of the present specification, "[s]urprisingly, these complexes, devoid of any other host or microbial molecules, are potent and water soluble, not requiring additional lipid carrier molecules (*e.g.*, serum albumin) for water solubility." Further, page 18 of the specification extensively discusses the surprising results made by the inventors. Page 18, lines 2-9 state that the "complex can be generated with about physiologic (pM) concentrations of endotoxin and soluble MD-2. This complex, at pg/ml concentrations, activates cells in a TLR4-dependent fashion without the inclusion of other host or bacterial factors. The surprising and unexpected success in achieving formation of a bioactive endotoxin-MD-2 complex at such low concentrations of endotoxin and MD-2 reflects the importance of presenting endotoxin to MD-2 after endotoxin has been first modified by LBP and CD14." Page 18, line 16 states that that "a greater reactivity of MD-2 for disaggregated vs. aggregated forms of endotoxin" allows for the formation of the claimed monomeric complex. Thus, the present claimed invention was surprising and unexpected in view of the state of the art at the time the application was filed.

Thus, since Viriyakosol et al. do not teach or suggest all of the features of the claimed invention, Viriyakosol et al. does not anticipate the claimed invention. Further, the resulting product, the claimed monomeric MD-2:endotoxin complex was a surprising and unexpected result given the teachings at the time the application was filed. Accordingly, Applicant respectfully requests withdrawal of this 35 U.S.C. 102(b) rejection of the claims.

MD-2 Sequence

In a telephone conference with Examiner Audet on April 27, 2006, the Examiner requested the sequence of MD-2. Attached hereto is the nucleic acid and amino acid sequence of wild-type MD-2.

During the telephone conference, the Examiner also inquired if it was known where on MD-2 the endotoxin was bound in the monomeric complex. This is not yet published in the literature.

Allowable Subject Matter

The Examiner has indicated that claims 11-19 are free of the art. These claims were objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the features of the base claim and any intervening claims. These claims have been amended as suggested by the Examiner. Applicants respectfully request that this objection be withdrawn.

CONCLUSION

Applicants respectfully submit that the claims are in condition for allowance, and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicants' attorney at (952) 876-4091 to facilitate prosecution of this application.

If necessary; please apply any charges or credits to Deposit Account No. 50-3503.

Respectfully submitted,

JERROLD P. WEISS et al.

By their Representatives,

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Date: 27 April 2006

By: _____
Ann S. Viksnins
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Nucleic Acid Sequence of Human MD-2

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  1 taaatctttt ctgcttactg aaaaggaaga gtctgatgat tagttactga tcctctttgc
 61 atttgtaaag ctttggagat attgaatcat gttaccattt ctgtttttt ccaccctgtt
121 ttcttccata tttactgaag ctcagaagca gtattgggtc tgcaactcat ccgatgcaag
181 tatttcatac acctactgtg ataaaatgca atacccaatt tcaattaatg ttaacccctg
241 tatagaattg aaaggatcca aaggattatt gcacattttc tacattccaa ggagagattt
301 aaagcaatta tatttcaatc tctatataac tgtcaacacc atgaatcttc caaagcgcaa
361 agaagttatt tgccgaggat ctgatgacga ttactctttt tgcagagctc tgaagggaga
421 gactgtgaat acaacaatat cattctcctt caagggaata aaattttcta agggaaaata
481 caaatgtgtt gttgaagcta tttctgggag cccagaagaa atgctctttt gcttggagtt
541 tgtcatccta caccaaccta attcaaatta gaataaattg agtattt
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Corresponding Amino Acid Sequence of Human MD-2

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  1 mlpflffstl fssifteaqk qywvcnssda sisytycdkm qypisinvnp cielkgskgl
 61 lhifyiprrd lkqlyfnlyi tvntmnlpkr kevicrgsdd dysfcralkg etvnttisfs
121 fkgikfskgk ykcvveaisg speemlfcle fvilhqpnsn
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